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Part IV

Department of Health and Human Services

Food and Drug Administration

21 CFR Part 184

Direct Food Substances Affirmed as Generally Recognized as Safe; Chymosin Enzyme Preparation Derived From Escherichia Coli K-12; Final Rule

Pfizer Central Research, Pfizer, Inc.; Withdrawal of Food Additive Petition; Notice

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 184

[Docket Nos. 87G-0418 and 87F-0416]

Direct Food Substances Affirmed as Generally Recognized as Safe; Chymosin Enzyme Preparation Derived From Escherichia Coli K-12

AGENCY: Food and Drug Administration.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is amending its regulations to affirm that the use of chymosin preparation derived by fermentation from *Escherichia coli* K-12 is generally recognized as safe (GRAS). This action is in response to a petition filed by Pfizer Central Research, Pfizer, Inc.

EFFECTIVE DATE: March 23, 1990. The Director of the Office of the Federal Register approves the incorporation by reference in accordance with 5 U.S.C. 552(a) of a certain publication in 21 CFR 184.1685(b) effective on March 23, 1990.

FOR FURTHER INFORMATION CONTACT: Eric L. Flamm, Center for Food Safety and Applied Nutrition (HFF-334), Food and Drug Administration, 200 C Street SW., Washington, DC 20204, 202-426-8950.

SUPPLEMENTARY INFORMATION:

I. Background

In accordance with the procedures described in § 170.35 (21 CFR 170.35), Pfizer Central Research (Pfizer) submitted a petition (GRASP 8G0337) requesting that its chymosin preparation (referred to as "chymosin" in the petition's filing notice that FDA published in the **Federal Register** of February 9, 1988 (53 FR 3792)), which is derived from the fermentation of a genetically modified *E. coli* K-12, be affirmed as GRAS as a direct human food ingredient. Chymosin is the principal enzyme in rennet, a GRAS food ingredient used for its milk-clotting activity, and is primarily responsible for that activity. Chymosin preparation is intended for use as a substitute for rennet.

To avoid confusion between chymosin the enzyme and chymosin the enzyme preparation (in which chymosin is the principal active component but which may also contain impurities), this document will henceforth use the term "chymosin" when referring to the enzyme and "chymosin preparation" when referring to the fermentation-derived chymosin enzyme preparation.

Pfizer submitted two petitions concerning chymosin preparation. In addition to GRASP 8G0337, it submitted a petition (FAP 8A4048) requesting that the food additive regulations be amended to provide for the safe use of genetically modified *E. coli* K-12 as a source of chymosin preparation (referred to as "chymosin" in the petition's filing notice that FDA published in the **Federal Register** of February 9, 1988 (53 FR 3792)), for use in food. The regulation that is the subject of this final rule is in response to the GRAS affirmation petition. Published elsewhere in this issue of the **Federal Register** is a document (Docket No. 87F-0416) announcing that Pfizer has withdrawn its food additive petition without prejudice to a future filing.

FDA gave interested parties an opportunity to submit comments concerning the GRAS affirmation petition to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857. FDA received two comments in response to the notice. Both comments address whether the dairy industry needs new sources of chymosin. Neither comment contains any information relevant to the safety, functionality, environmental impact, or GRAS status of the food use of the subject chymosin preparation. Thus, the comments are not relevant to the agency's evaluation of chymosin preparation. FDA's authority in reviewing food additive and GRAS affirmation petitions is limited to questions about the safety and functionality of the substance at issue and does not include questions about the need for a new food ingredient (21 U.S.C. 348).

II. Standards for GRAS Affirmation

Pursuant to § 170.30 (21 CFR 170.30), general recognition of safety may be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, experience based on common use in food. General recognition of safety based upon scientific procedures requires the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation and ordinarily is to be based upon published studies, which may be corroborated by unpublished studies and other data and information (§ 170.30(b)). In its petition, Pfizer has relied upon scientific procedures to establish that chymosin preparation is GRAS.

Rennet is an animal-derived enzyme preparation that is GRAS as specified in § 184.1685 (21 CFR 184.1685). Therefore, if published information shows that the principal active component of chymosin preparation is the same as that of rennet, and that the other components (i.e., impurities) of the chymosin preparation, which may differ from the other components (i.e., impurities) of rennet, do not render the use of the substance unsafe, then chymosin preparation derived from *E. coli* would present no more safety concern than rennet. If this is the case, FDA can affirm chymosin preparation derived from *E. coli* as GRAS for use as a replacement for rennet.

III. Safety

A. Introduction

Chymosin, also known as rennin, is the principal milk-clotting enzyme present in rennet (Ref. 1). Rennet is an enzyme preparation that will clot milk, forming it into curds and whey (Refs. 1 and 2). It is used to make cheese and other dairy products. It has a long and extensive history of safe use in food and has been affirmed by FDA as GRAS in § 184.1685 (48 FR 51151; November 7, 1983).

Food-grade rennet is an enzyme preparation that is derived from the fourth stomach of calves, kids, or lambs. Commercially, it is generally derived from unweaned calves by aqueous extraction. The aqueous extraction step is followed by purification steps and an acidification step to cleave prochymosin (the inactive precursor of chymosin) in the rennet into chymosin (Ref. 1).

There are two predominant forms of calf chymosin, chymosin A and chymosin B (Ref. 1). Foltmann et al. have shown that chymosin A and chymosin B differ by a single amino acid (Ref. 3). In this document FDA is using the terms "chymosin" to refer to either, or both, chymosin A and chymosin B.

Techniques developed in the last 15 years (frequently termed "recombinant DNA technology" or "cloning techniques") enable scientists to locate and to obtain a segment of deoxyribonucleic acid (DNA) containing a gene of interest. They are able to move that DNA segment into a vector (a self-replicating DNA molecule that is easy to manipulate) and then introduce it into a new host organism where it can be correctly expressed (that is, produce the protein that it would produce in the original organism). These techniques are

well-known to molecular biologists (see e.g., Refs. 4 and 5).

B. The Chymosin Component

Using cloning techniques, scientists in a number of different laboratories have identified the gene in the calf from which the chymosin in rennet is produced, the prochymosin gene (Refs. 6 through 8). Scientists have moved the calf prochymosin gene into *E. coli* (Refs. 6 through 10) as well as into other microorganisms (Refs. 11 through 13).

They have used a variety of techniques to demonstrate that they have cloned full-length copies of the correct gene. Such techniques include: (1) DNA sequencing, whereby the cloned putative prochymosin gene was shown to have the nucleotide sequence that encodes the amino acid sequence of prochymosin (Refs. 6 through 8); (2) nucleic acid hybridization, whereby the cloned DNA fragments or the ribonucleic acid (RNA) molecules made from the DNA fragments were shown to hybridize (i.e., specifically bind) with complementary DNA in the prochymosin gene (Refs. 7 through 12); and (3) physical mapping, whereby the cloned DNA fragments were shown to be large enough to contain the prochymosin gene and, when digested with appropriate DNA-cutting enzymes and run on gels that separate DNA fragments by size, were shown to yield the pattern of DNA fragments expected for prochymosin (Refs. 7 through 13).

The published evidence establishes that the new host organisms are able to use the prochymosin gene to produce prochymosin that has the same molecular weight as prochymosin found in calf rennet (Refs. 9 through 11 and 13 through 15). It also establishes that the prochymosin that is produced (cloned prochymosin) can be cleaved into chymosin (cloned chymosin) that has the same molecular weight and the same functional activity as chymosin found in calf rennet (Refs. 9, 10, and 12 through 15).

The molecular weights of prochymosin and chymosin were assayed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, a technique that enables one to determine the comparative molecular weight of proteins based on their rate of migration through the gel. Cloned prochymosin was found to migrate through these gels at the same rate as the prochymosin derived from calves (Refs. 9 through 11 and 13 through 15). Cloned chymosin was found to migrate through these gels at the same rate as the chymosin found in rennet (Refs. 9, 10, and 12 through 15).

The functional activity of chymosin that was measured was milk-clotting activity. Cloned chymosin was found to clot milk at the same rate as the chymosin in rennet under various temperatures, salt concentrations, and pH conditions (Refs. 9 through 15).

One safety concern raised by cloning is whether extraneous DNA, particularly DNA flanking the gene of interest, that could potentially encode extraneous harmful proteins may be cloned along with the gene of interest (i.e., prochymosin). However, the regulation stipulates that the substance being affirmed as GRAS is one that is produced using a production strain that is nontoxigenic (see § 184.1685(a)(2)). If the cloned DNA encodes a harmful substance that could render the enzyme preparation unsafe, the production strain would be toxigenic, and the substance produced would not be GRAS under § 184.1685(a)(2). Therefore, the agency finds that there is no basis for concern that the safety of the chymosin preparation will be compromised by contaminating proteins encoded by extraneous uncharacterized DNA cloned along with the prochymosin gene.

FDA notes that as a matter of good manufacturing practice, manufacturers using recombinant DNA technology should assure themselves that they have not inadvertently cloned extraneous protein-encoding DNA along with the prochymosin gene. Such assurance can come from reviewing the details of the cloning steps, such as the origin and sequence of the DNA fragments used in the cloning, and from full characterization of the final genetic constructs via techniques such as DNA sequencing. The agency points out that Pfizer's petition contains information demonstrating that it conducted these steps.

Based on the fact that published information demonstrates that chymosin produced from the cloned prochymosin gene has the same molecular weight and the same functional activity as the chymosin derived from calves, FDA concludes that the chymosin enzyme in chymosin preparation is the same as the chymosin enzyme in calf rennet. Therefore, FDA concludes that the chymosin enzyme in chymosin preparation is as safe as the chymosin enzyme in rennet.

C. Sources of Impurities

Enzyme preparations used in food-processing are usually not chemically pure but contain extraneous source (cellular) and processing material. The nature and amounts of these impurities in the finished enzyme preparation depend on the organism from which the enzyme is produced (the source or production organism), the fermentation materials and methods used to grow the production organism, and the materials and methods used to generate the finished enzyme preparation.

Both the source material and the manufacturing methods for producing chymosin preparation differ from those used to produce animal rennet. Therefore, the impurities in chymosin preparation will differ from those in rennet. The question thus is whether the source material or manufacturing methods for chymosin preparation will introduce impurities that would raise concerns about the safety of the preparation.

1. Processing steps

Researchers in a number of laboratories have published papers containing descriptions of methods that they used for producing chymosin preparation from microorganisms containing the calf prochymosin gene (e.g., Refs. 9 through 15). The methods described in these publications and in Pfizer's petition do not differ from each other in any significant way. The key steps in the methods, as described by Marston et al. (Ref. 15) and by Pfizer, are summarized below.

E. coli is grown in a liquid nutrient medium, then pelleted by centrifugation. The pelleted cells are resuspended in a small volume of buffer and burst open, releasing the prochymosin in the form of densely packed insoluble aggregates. The aggregates are recovered by a second centrifugation step, while most of the rest of the cellular protein and debris remain in suspension. The prochymosin is thus rapidly purified from the bulk of cellular material. Pfizer treats the aggregates with acid to kill residual cells and to degrade residual DNA. The prochymosin aggregates are solubilized by denaturation in an alkaline urea solution. The prochymosin is renatured by decreasing the alkalinity and diluting out the urea and further purified by ion-exchange chromatography. The prochymosin is converted to chymosin, the active form of the enzyme, by suspension in a pH 2 acid solution for an hour. The final chymosin preparation is a clear solution.

FDA finds that the manufacturing method does not require the use of any processing materials that are not GRAS or approved food additives. Accordingly, it is specifying in the regulation that the substance being affirmed as GRAS is one that is produced using only processing materials that are GRAS substances or food additives approved for use in this type of process (see § 184.1685(a)(2)). Therefore, the agency concludes that the

manufacturing steps will not introduce impurities into the enzyme preparation that will adversely affect the safety of the preparation.

2. Production organism

The source material for the chymosin in chymosin preparation is *E. coli* K–12. *E. coli's* natural habitat is the large intestine of humans and animals, where it is found at 1 million to 100 million organisms per gram of intestinal contents (Ref. 16). *E. coli* K–12 is a laboratory strain that, through longtime propagation in the laboratory, has lost a number of the traits necessary for colonization of, and therefore survival in, the intestinal tract (Ref. 17).

E. coli K–12 is one of biology's most extensively studied microorganisms. It has been shown to be nonpathogenic and nontoxigenic in studies in which it has been fed to humans and laboratory animals (see, e.g., Refs. 16 and 17) and in detailed studies of its phenotypic and genotypic traits (see, e.g., Refs. 16 through 18). For example, Gorbach (Ref. 16), Curtiss (Ref. 17), and Smith (Ref. 18) have reported their own and others' failed attempts to implant *E. coli* K–12 in the human intestine, an initial step for pathogenicity. In none of the experiments were researchers able to isolate the strain from the feces of volunteers 1 to 6 days after they were fed high doses (10^9–10^{10} live organisms) of various strains of *E. coli* K–12. In none of the experiments did the volunteers become sick as a result of their ingestion of the organisms.

Gorbach (Ref. 16) discussed six factors necessary for an organism to be pathogenic: (1) Survival in the environment, (2) a mechanism for penetrating the skin or mucosal surface, (3) multiplication within the host, (4) systemic spread within the host, (5) resistance to host defense mechanisms, and (6) production of a toxin or some other mechanism to damage the host to produce disease symptoms. He noted that a lack of any one of these characteristics will render the microorganism nonpathogenic, and that, based on the available evidence, *E. coli* K–12 is deficient in every one of these factors (Ref. 16). Gorbach also noted that in the 30 years that *E. coli* K–12 has been used in genetics research, there have been no reported cases of laboratory-acquired infections from this organism (Ref. 16).

As corroborative evidence of the safety of chymosin preparation, Pfizer submitted two unpublished short-term in vivo studies conducted on its enzyme preparation, a 5-day feeding study in dogs and a 1-month gavage study in rats (Ref. 19). No adverse effects were observed in these studies at any dose fed.

Some *E. coli* K–12 strains, such as those that are used by Pfizer and others (e.g., Refs. 9, 10, and 14) to produce chymosin preparation, do contain marker genes that encode resistance to clinically useful antibiotics. Such genes potentially could be transferred to other microorganisms with which the production strain or its DNA comes into contact. However, as previously described, the isolation of the enzyme as an intracellular insoluble aggregate results in the destruction of the microorganism and in the elimination of most cellular material, including these marker genes (Ref. 15). Additionally, the two acid treatment steps in the manufacturing process inactivate residual cells and degrade residual DNA, including marker genes, that remain in the enzyme preparation (Ref. 20).

As corroborative evidence that the enzyme preparation does not contain gene-size DNA fragments or transformable DNA (that is, DNA that a microorganism can take up from its surroundings and functionally incorporate into its own DNA), Pfizer submitted data from several unpublished experiments, including a gel electrophoresis/DNA hybridization assay and a transformation assay. In the electrophoresis experiment, DNA fragments were sized based on their differential rates of migration through the gel and quantitated based on their level of hybridization with labeled complementary DNA. No DNA fragments large enough to contain an intact gene encoding antibiotic resistance were detected in the enzyme preparation (Ref. 19).

In the transformation assay, bacterial cells were mixed with DNA under optimized conditions and assayed to see if they had picked up the antibiotic resistance encoded by the DNA. Cells mixed with the enzyme preparation did not become antibiotic resistant (Ref. 19).

Based on the above discussion, FDA concludes that chymosin preparation manufactured in conformity with § 184.1685(a)(2) will not contain DNA encoding resistance to antibiotics at levels that would provide any safety concern. FDA concludes that *E. coli* K–12 is safe for use as a source of food-grade chymosin preparations, and that impurities resulting from its use in the production of chymosin preparation will not affect the safety of the chymosin preparation.

IV. Specifications

The agency finds that, because the principal active ingredient of chymosin preparation and rennet are the same, and because the impurities in chymosin preparation do not provide any basis for concern that the use of the preparation may not be safe, the general and additional requirements given for rennet and other enzyme preparations in the "Food Chemicals Codex," 3d Ed. (1981), pp. 107–110, are adequate for defining minimum criteria for a food-grade chymosin preparation derived from *E. coli* K–12. FDA is amending § 184.1685(b) to reference pp. 109 and 110 of the "Food Chemicals Codex," 3d Ed. (1981), as well as pp. 107 and 108.

V. Conclusions

The agency has evaluated all available information and finds, based upon the published and corroborative evidence discussed above, that the active principal ingredient in the chymosin preparation is the same as that in rennet, and that when the preparation is manufactured in accordance with § 184.1685(a)(2), the source organism and manufacturing process will nor introduce impurities into the preparation that would provide a basis for concern that the use of the preparation may not be safe. Therefore, the agency concludes, based upon scientific procedures, that the chymosin preparation derived by fermentation from *E. coli* K–12 and described in the regulation below is GRAS for use as a replacement for rennet.

VI. Environmental Effects

The agency has carefully considered the potential environmental effects of this action and has concluded that the action will not have a significant impact on the human environment and that an environmental impact statement is not required. The agency's finding of no significant impact and the evidence supporting that finding, contained in an environmental assessment, may be seen in the Dockets Management Branch (address above).

VII. Economic Effects

FDA, in accordance with the Regulatory Flexibility Act, has considered the effects that this regulation would have on small entities, including small businesses, and has determined that the effect of this regulation is to provide for the use of fermentation-derived chymosin for both large and small businesses. Therefore, FDA certifies in accordance with section 605(b) of the Regulatory Flexibility Act that no significant economic impact on a substantial number of small entities will derive from this action.

In accordance with Executive Order 12291, the agency has analyzed the economic effects of this final rule and has determined that this rule will not be a major rule as defined by that Order.

The agency's finding of no major economic impact and no significant impact on a substantial number of small entities, and the evidence supporting these findings, are contained in a threshold assessment which may be seen in the Dockets Management Branch (address above).

VIII. References

The following references have been placed on display in the Dockets Management Branch (address above) and may be seen by interested persons between 9 a.m. and 4 p.m., Monday through Friday.

1. Foltmann, B., "Prochymosin and Chymosin (Prorennin and Rennin)," *Methods in Enzymology*, 19:421–436, 1970.
2. Burgess, K., and M. Shaw, "Dairy," in *"Industrial Enzymology,"* T. Godfrey and J. Reichelt, eds., The Nature Press, New York, pp. 260–265, 1983.
3. Foltmann, B. et al., "The Primary Structure of Calf Chymosin," *Journal of Biological Chemistry*, 254:8447–8456, 1979.
4. Watson, J.D. et al., *"Molecular Biology of the Gene,"* Benjamin/Cummings Publishing Co., Inc., Menlo Park, CA, pp. 65–94, 202–213, 1987.
5. Murray, K., "Genetic Engineering and its Applications," in *"Basic Biotechnology,"* J. D. Bu'Lock and B. Kristiansen, eds., Academic Press, London, pp. 483–508, 1987.
6. Harris, T. J. R. et al., "Molecular Cloning and Nucleotide Sequence of cDNA Coding for Calf Preprochymosin," *Nucleic Acids Research*, 10:2177–2187, 1982.
7. Hidaka, M. et al., "Cloning and Structural Analysis of the Calf Prochymosin Gene," *Gene*, 43:197–203, 1986.
8. Moir, D. et al., "Molecular Cloning and Characterization of Double-stranded cDNA Coding for Bovine Chymosin," *Gene*, 19:127–138, 1982.
9. Emtage, J.S. et al., "Synthesis of Calf Prochymosin (Prorennin) in *Escherichia coli*," *Proceedings of the National Academy of Sciences*, 80:3671–3675, 1983.
10. McCaman, M.T., W.H. Andrews, and J.G. Files, "Enzymatic Properties and Processing of Bovine Prochymosin Synthesized in *Escherichia coli*," *Journal of Biotechnology*, 2:177–190, 1985.
11. Goff, C.G. et al., "Expression of Calf Prochymosin in *Saccharomyces cerevisiae*," *Gene*, 27:35–46, 1984.
12. Cullen, D. et al., "Controlled Expression and Secretion of Bovine Chymosin in *Aspergillus nidulans*," *Bio/Technology*, 5:369–376, 1987.
13. Mellor, J. et al., "Efficient Synthesis of Enzymatically Active Calf Chymosin in *Saccharomyces cerevisiae*," *Gene*, 24:1–14, 1983.
14. Kawaguchi, Y. et al., "Production of Chymosin in *Escherichia coli* Cells and its Enzymatic Properties," *Agricultural and Biological Chemistry*, 51:1871–1877, 1987.
15. Marston, F.A.O. et al., "Purification of Calf Prochymosin (Prorennin) in *Escherichia coli*," *Bio/Technology*, 2:800–804, 1984.
16. Corbach, S.L., "Recombinant DNA: An Infectious Disease Perspective," *Journal of Infectious Diseases*, 137:615–623, 1978.
17. Curtiss, R., "Biological Containment and Cloning Vector Transmissibility," *Journal of Infectious Diseases*, 137:668–675, 1978.
18. Smith, H.W., "Is It Safe to Use *Escherichia coli* K–12 in Recombinant DNA Experiments?," *Journal of Infectious Diseases*, 137:655–660, 1978.
19. Petition 8C0337.
20. Lehninger, A.L., *"Biochemistry,"* Worth Publishers, New York, p. 256, 1970.

List of Subjects in 21 CFR Part 184

Food ingredients, Incorporation by reference.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR part 184 is amended as follows:

PART 184—DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE

1. The authority citation for 21 CFR part 184 continues to read as follows:

Authority: Secs. 201, 402, 409, 701 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 342, 348, 371).

2. Section 184.1685 is revised to read as follows:

§ 184.1685 Rennet (animal-derived) and chymosin preparation (fermentation-derived).

(a) (1) Rennet and bovine rennet are commercial extracts containing the active enzyme rennin (CAS Reg. No. 9001–98–3), also known as chymosin (International Union of Biochemistry Enzyme Commission (E.C.) 3.4.23.4). Rennet is the aqueous extract prepared from cleaned, frozen, salted, or dried fourth stomachs (abomasa) of calves, kids, or lambs. Bovine rennet is the product from adults of the animals listed above. Both products are called rennet and are clear amber to dark brown liquid preparations or white to tan powders.

(2) Chymosin preparation is a clear solution containing the active enzyme chymosin (E.C. 3.4.23.4). It is derived, via fermentation, from a nonpathogenic and nontoxigenic strain of *Escherichia coli* K–12 containing the prochymosin gene. The prochymosin is isolated as an insoluble aggregate that is acid-treated to destroy residual cellular material and, after solubilization, is acid-treated to form chymosin. It must be processed with materials that are generally recognized as safe, or are food additives that have been approved by the Food and Drug Administration for this use.

(b) Rennet and chymosin preparation meet the general and additional requirements for enzyme preparations of the "Food Chemicals Codex," 3d Ed. (1981), pp. 107–110, which is incorporated by reference in accordance with 5 U.S.C. 552(a). Copies are available from the National Academy Press, 2101 Constitution Avenue NW., Washington, DC 20418, or are available for inspection at the Office of the Federal Register, 1100 L Street NW., Washington, DC.

(c) In accordance with § 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as an enzyme as defined in § 170.3(o)(9) of this chapter; a processing aid as defined in § 170.3(o)(24) of this chapter; and a stabilizer and thickener as defined in § 170.3(o)(28) of this chapter.

(2) The ingredient is used in the following foods at levels not to exceed current good manufacturing practice: In cheeses as defined in § 170.3(n)(5) of this chapter; frozen dairy desserts and mixes as defined in § 170.3(n)(20) of this chapter; gelatins, puddings, and fillings as defined in § 170.3(n)(22) of this chapter; and milk products as defined in § 170.3(n)(31) of this chapter.

(d) Prior sanctions for this ingredient different from the uses established in this section do not exist or have been waived.

Dated: March 14, 1990.

Alan L. Hoeting,

Acting Associate Commissioner for Regulatory Affairs.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

[Docket No. 87F-0416]

Pfizer Central Research, Pfizer, Inc.; Withdrawal of Food Additive Petition

AGENCY: Food and Drug Administration.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing the withdrawal, without prejudice to future filing, of a petition (FAP 8A4048) proposing that the food additive regulations be amended to provide for the safe use of a genetically modified *Escherichia coli* K–12 (*E. coli* K–12) as a source of chymosin for use in food.

FOR FURTHER INFORMATION CONTACT: Eric L. Flamm, Center for Food Safety and Applied Nutrition (HFF–334), Food and Drug Administration, 200 C Street SW., Washington, DC 20204, 202–426–8950.

SUPPLEMENTARY INFORMATION: In the **Federal Register** of February 9, 1988 (53 FR 3792), FDA published a notice that it had filed a petition (FAP 8A4048) from Pfizer Central Research, Pfizer, Inc., 235 East 42d St., New York, NY 10017, that proposed to amend the food additive regulations to provide for the safe use of a genetically modified *E. coli* K–12 as a source of prochymosin.

The prochymosin preparation obtained by fermentation of the modified *E. coli* K–12 is processed to yield chymosin for use in food. Pfizer Central Research, Pfizer, Inc., has now withdrawn the petition without prejudice to a future filing (21 CFR 171.7). Published elsewhere in this issue of the **Federal Register** is a final rule (Docket No. 87G–0418) affirming that the use of chymosin preparation derived from *E. coli* K–12 is generally recognized as safe.

Dated: March 14, 1990.

Alan L. Hoeting,

Acting Associate Commissioner for Regulatory Affairs.

[FR Doc. 90–6599 Filed 3–22–90; 8:45 am]

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